### Order information

<table>
<thead>
<tr>
<th>LDL-Cholesterol plus 2nd generation</th>
<th>System-ID</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>175 tests</td>
<td>03038866</td>
<td>322</td>
</tr>
<tr>
<td>Calibrator f.a.s. Lipids (3 x 1 mL)</td>
<td>12172623</td>
<td>122</td>
</tr>
<tr>
<td>Calibrator f.a.s. Lipids (3 x 1 mL, for USA)</td>
<td>12172623</td>
<td>160</td>
</tr>
<tr>
<td>Precinorm L (4 x 3 mL)</td>
<td>10781827</td>
<td>122</td>
</tr>
<tr>
<td>Precipath HDL/LDL-C (4 x 3 mL)</td>
<td>11778552</td>
<td>122</td>
</tr>
<tr>
<td>Diluent NaCl 9 % (50 mL)</td>
<td>04489357</td>
<td>190</td>
</tr>
</tbody>
</table>

### Method

The method for cholesterol determination (cholesterol esterase, cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL < LDL. In the presence of Mg++, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum. 1,2,7,8

Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.9,10,11

This direct assay meets the 1995 NCEP goals of < 4 % total CV, bias ≤ 4 % versus reference method, and ≤ 12 % total analytical error.12

### Test principle

Homogeneous enzymatic colorimetric assay.

- LDL-cholesterol esters + H₂O → Cholesterol esterase → Cholesterol + free fatty acids (selective micellary solubilization)
- Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.
- LDL-Cholesterol + O₂ → Cholesterol oxidase → Δ⁴-cholestenone + H₂O₂
- In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ⁴-cholestenone and hydrogen peroxide.
- 2 H₂O₂ + 4-aminoantipyrine + HSO₄⁻ + H₂O → Peroxidase → Purple-blue pigment + 5 H₂O (Abs. max = 585 nm)

### Reagents - working solutions

- **R1** MOPS (3-morpholinopropan sulfonic acid) buffer: 20.1 mmol/L, pH 6.5; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp., recombinant): ≥ 50 μkat/L; peroxidase (horseradish): ≥ 167 μkat/L; preservative
- **R2** MOPS (3-morpholinopropan sulfonic acid) buffer: 20.1 mmol/L, pH 6.8; MgSO₄·7H₂O: 8.11 mmol/L; 4-aminoantipyrine: 2.46 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥ 50 μkat/L; cholesterol oxidase (Brevibacterium spec., recombinant): ≥ 33.3 μkat/L; peroxidase (horseradish): ≥ 334 μkat/L; detergent; preservative

### Precautions and warnings

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines.

### Reagent handling

Ready for use.
**LDL_C**

**LDL-Cholesterol plus 2nd generation**

**Storage and stability**

**LDL_C**
- Shelf life at 2-8 °C: See expiration date on cobas c pack label.
- On-board in use and refrigerated on the analyzer: 12 weeks

**Diluent NaCl 9%**
- Shelf life at 2-8 °C: See expiration date on cobas c pack label.
- On-board in use and refrigerated on the analyzer: 12 weeks

**Specimen collection and preparation**
- For specimen collection and preparation, only use suitable tubes or collection containers.
- Only the specimens listed below were tested and found acceptable.
- Plasma: Li-heparin plasma
- EDTA plasma causes decreased values.

**Materials provided**
- See “Reagents - working solutions” section for reagents.

**Materials required (but not provided)**
- See “Order information” section.
- General laboratory equipment

**Assay**
- For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

**Application for serum and plasma**

**cobas c 311 test definition**

- **Assay type**: 2 Point End
- **Reaction time / Assay points**: 10 / 6-31
- **Wavelength (sub/main)**: 700/600 nm
- **Reaction direction**: Increase
- **Units**: mmol/L (mg/dL, g/L)
- **Reagent pipetting**: Diluent (H₂O)
- **R1**: 150 µL
- **R2**: 50 µL
- **Sample volumes**: Sample
- **Sample dilution**: Sample
- **Sample Diluent (NaCl)**
  - **Normal**: 2 µL
  - **Decreased**: 10 µL 15 µL 135 µL
  - **Increased**: 4 µL

**cobas c 501/502 test definition**

- **Assay type**: 2 Point End
- **Reaction time / Assay points**: 10 / 10-47
- **Wavelength (sub/main)**: 700/600 nm
- **Reaction direction**: Increase
- **Units**: mmol/L (mg/dL, g/L)
- **Reagent pipetting**: Diluent (H₂O)
- **R1**: 150 µL
- **R2**: 50 µL
- **Sample volumes**: Sample
- **Sample dilution**: Sample
- **Sample Diluent (NaCl)**
  - **Normal**: 2 µL
  - **Decreased**: 10 µL 15 µL 135 µL
  - **Increased**: 4 µL

**Calibration**

- **Calibrators**: S1: H₂O
  - **S2**: C.f.a.s. Lipids
- **Calibration mode**: Linear
- **Calibration frequency**: 2-point calibration
  - after reagent lot change
  - and as required following quality control procedures

**Traceability**: This method has been standardized against the beta quantification method as defined in the recommendations in the LDL Cholesterol Method Certification Protocol for Manufacturers.³

**Quality Control**

- For quality control, use control materials as listed in the “Order information” section.
- Other suitable control material can be used in addition.
- The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
- Follow the applicable government regulations and local guidelines for quality control.

**Calculation**

- Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

**Conversion factors**: mmol/L x 38.66 = mg/dL
- mmol/L x 0.3866 = g/L
- mg/dL x 0.0259 = mmol/L

**Limitations – interference**

- **Criterion**: Recovery within ± 10 % of initial values at LDL cholesterol levels of 4.0 mmol/L (154 mg/dL).
- **Icterus**: No significant interference up to an l index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).
- **Hemolysis**: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).
- **Lipemia (IntraLipid)**: No significant interference up to an L index of 200.
- **Drugs**: No interference was found at therapeutic concentrations using common drug panels.²
- **Exception**: IntraLipid causes artificially high LDL cholesterol results.
- **Ascorbic acid** up to 50 mg/dL (2.84 mmol/L) does not interfere.
- Abnormal liver function affects lipid metabolism; consequently HDL and LDL results are of limited diagnostic value. In some patients...
with abnormal liver function, the LDL-cholesterol result is significantly negatively biased versus beta quantification results.

In very rare cases, gammapathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

**ACTION REQUIRED**

**Special Washing Programming:** The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. The latest version of the Carry over evasion list can be found with the NaOH/SDS/Multiclean/SCCS or the NaOH/SDS/SmpCln + 1/2SCCS Method Sheets. For further instructions refer to the operator manual.

**cobas c 502 analyzer:** All special wash programming necessary for avoiding carry over is available via the cobas link, manual input is not required.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

**Limits and ranges**

- **Measuring range:** 0.10-14.2 mmol/L (3.86-548 mg/dL)
- Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2.

**Lower limits of measurement**

- **Lower detection limit of the test:** 0.10 mmol/L (3.66 mg/dL)

The lower detection limit represents the lowest measurable analyte limit that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

**Expected values**

Levels in terms of risk for coronary heart disease:

- Adult levels:
  - Optimal: < 2.59 mmol/L (< 100 mg/dL)
  - Near optimal/above optimal: 2.59-3.34 mmol/L (100-129 mg/dL)
  - Borderline high: 3.37-4.12 mmol/L (130-159 mg/dL)
  - High: 4.14-4.89 mmol/L (160-189 mg/dL)
  - Very high: ≥ 4.92 mmol/L (≥ 190 mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

**Specific performance data**

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

**Precision**

Precision was determined using human samples and controls in an internal protocol. Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

<table>
<thead>
<tr>
<th>Repeatability *</th>
<th>Mean mmol/L (mg/dL)</th>
<th>SD mmol/L (mg/dL)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precinorm L</td>
<td>2.78 (107)</td>
<td>0.02 (1)</td>
<td>0.7</td>
</tr>
<tr>
<td>Precipath HDL/LDL-C</td>
<td>5.50 (212)</td>
<td>0.04 (2)</td>
<td>0.8</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>2.51 (96.9)</td>
<td>0.02 (0.8)</td>
<td>0.9</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>6.14 (237)</td>
<td>0.08 (3)</td>
<td>1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intermediate precision **</th>
<th>Mean mmol/L (mg/dL)</th>
<th>SD mmol/L (mg/dL)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precinorm L</td>
<td>2.65 (102)</td>
<td>0.07 (3)</td>
<td>2.7</td>
</tr>
<tr>
<td>Precipath HDL/LDL-C</td>
<td>5.42 (209)</td>
<td>0.12 (5)</td>
<td>2.3</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>1.47 (56.8)</td>
<td>0.03 (1.2)</td>
<td>1.9</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>3.95 (153)</td>
<td>0.08 (3)</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* repeatability = within-run precision
** Intermediate precision = total precision / between run precision / between day precision

**Method comparison**

LDL cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared to those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 171

- **Passing/Bablok**
  - Linear regression
  
  \[
  y = 0.973x + 0.143 \text{ mmol/L} \\
  r = 0.993 \text{ mmol/L} \\
  \tau = 0.940
  \]

The sample concentrations were between 1.28 and 12.8 mmol/L (48.6 and 494 mg/dL).

**References**

12. Data on file at Roche Diagnostics.
LDL-C
LDL-Cholesterol plus 2nd generation

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